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Transmission and latency of cherry necrotic ring spot virus in Prunus tomentosa

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TRANSMISSION AND LATENCY OF CHERRY NECROTIC RING SPOT VIRUS IN *PRUNUS TOMENTOSA*

by

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INTRODUCTION

Necrotic ring spot virus is widely distributed in Prunus species and is always found in sour cherry trees infected with cherry yellows. The effects of necrotic ring spot virus on the health of cherry trees has not been accurately assessed; however, the cherry yellows disease is devastating, often reducing yields as much as 50 per cent. Freedom from necrotic ring spot in cherry trees assures freedom from yellows. Efforts for control of necrotic ring spot and yellows have been directed primarily towards obtaining virus-free stocks and scions so that trees produced in the nursery are virus-free.

Southwest Iowa nurseries produce large numbers of trees for the cherry industry. Early studies of the necrotic ring spot disease in these nurseries were directed towards obtaining a satisfactory index host to use under Iowa conditions. Index plants are required for detection of this virus, as it is latent in many hosts. As a result of this early work Fink (1950) found that P. tomentosa seedlings reliably expressed necrotic ring spot symptoms and could be used either for greenhouse or field indexing. Several investigators have confirmed Fink's view that P. tomentosa is a desirable index plant (Hobart, 1954; Fridlund, 1954b; Gilmer and Brase, 1956).

A positive necrotic ring spot reaction is indicated with P. tomentosa if an inserted bud causes characteristic symptoms

to develop. Moore and Keitt (1949) have shown that if latent necrotic ring spot virus is present in Montmorency cherry trees, insertion of infected buds will not cause symptom formation. Thus index plants which can contain necrotic ring spot virus in a latent condition could yield erroneous indexing results. This thesis reports on experiments which were designed to determine whether or not necrotic ring spot virus is latent in P. tomentosa.

The indexing required to assure the production of virus-free trees by nurseries has increased such that space and material for indexing are now of concern. This thesis reports on a new method of indexing with P. tomentosa which would not involve greenhouse space and also reports on the possibility of reusing P. tomentosa index plants.

Spread of necrotic ring spot virus has been shown to occur in sour cherry blocks in southwest Iowa nurseries by Hobart, Fink, and Buchholtz (1955). The low incidence of spread suggested a possible mechanical transmission. Experiments are reported here which were designed to examine the possibility of mechanical transmission.

REVIEW OF LITERATURE

The cherry yellows disease of sour cherry trees was determined to be of virus origin by Keitt and Clayton (1939). In studies of this disease they noticed necrotic ring spots develop prior to development of yellows symptoms on trees which had been inoculated with yellows infected buds. In the orchard they observed ring spot symptoms on trees which had never been observed to express yellows (Keitt and Clayton, 1943). Moore and Keitt (1944) showed that trees which were inoculated with buds from trees showing only necrotic ring spots developed only necrotic ring spots, but trees inoculated with buds from trees expressing both necrotic ring spots and yellows expressed both symptoms. Though necrotic ring spot virus can be found not associated with yellows, yellows infected trees have always been shown to contain necrotic ring spot virus. This relationship is not clear though some evidence indicates that the viruses responsible for the two diseases may be strains of the same virus. Ehlers (1957) has shown by heat inactivation studies with dormant budwood infected with both yellows and necrotic ring spot that the thermal inactivation end point of yellows parallels that of necrotic ring spot.

Necrotic ring spot symptoms have been observed in other Prunus species. Cochran and Hutchins (1941) inoculated

healthy peach trees with buds from orchard peach trees showing twig blight and severe dieback. The inoculated trees were slow in growth and initial leaves contained ring spots and mottled patterns. Later many leaves abscissed and subsequent foliage appeared normal. An indication that the virus causing necrotic ring spots in peach may be the same as the virus inducing necrotic ring spots in sour cherry was provided by Parker and Cochran (1951). They used buds from ring spot infected peach to inoculate Montmorency cherry trees and found that symptoms were produced which are identical to those usually produced with inoculum from necrotic ring spot infected sour cherry trees.

The initial effect of necrotic ring spot virus on Montmorency cherry is delayed foliation. Leaves are reduced in size and may show light-green spots and dark rings which vary from about one millimeter to five millimenters in diameter and have a watersoaked appearance. Later the affected areas may become necrotic and fall out. Symptom expression is usually limited to the first leaves unfolding. Generally trees severely affected one year show few or no symptoms in subsequent years (Berkeley et al., 1951).

This latent nature of necrotic ring spot virus has made it difficult to accurately assess the deleterious effects of this virus on sour cherry trees. Lewis (1951) compared the yield of necrotic ring spot infected sour cherry trees with healthy trees and found that 51 healthy trees averaged 177.3

pounds of fruit per tree, 40 trees each of which had expressed shock symptoms over the entire tree averaged 103.8 pounds, and 7 trees ring spot infected but not showing symptoms averaged 172.3 pounds per tree.

Klos (1954) found that the yield of necrotic ring spot infected Montmorency trees is reduced the first year of infection and in some cases the year following infection. Twelve trees infected in 1950, averaged 96.4 pounds of fruit per tree in 1950, 76.5 pounds in 1951, and 149.4 pounds in 1952. During the same three seasons, 11 healthy trees averaged 131.2, 133.3, and 144.1 pounds of fruit per tree.

Millikan and Hibbard (1952) determined the terminal growth of one-year-old Montmorency trees propagated from buds of a certified source and buds selected at random from nursery run sources. Terminal growth for certified trees averaged 50.2 inches and for the nursery run trees 42.6 inches. Millikan (1955) realizing that this difference in growth might have been attributable to buds infected with both yellows and necrotic ring spot, devised an experiment in which he compared the growth of one-year-old Montmorency trees propagated from certified budwood and from budwood of trees inoculated with buds infected with necrotic ring spot virus but free of yellows. The average height of infected and non-infected trees was approximately the same. Determinations of "average total growth" were made by summing all measurements of shoot growth that occurred on individual trees. An average of the

total shoot growth of 16 ring spot free trees was 224 inches; of trees inoculated with source G-2-1, 143.1 inches; and of trees inoculated with source B-1-12, 153.3 inches. Measurements of total growth were made approximately every two weeks beginning June 3, and ending August 16. There was little if any difference between diseased and healthy trees up to June 3, and late in the growing season, but significant differences were found during the middle of the growing season. Millikan attributed differences in growth to a greater amount of branching of ring-spot-free trees.

Milbrath (1950) has reported that the stand of buds in a row of Montmorency nursery stock has been reduced 50 per cent by using scionwood containing ring spot virus. Gilmer, Brase, and Parker (1957) determined the bud take of Montmorency cherry trees propagated with virus free buds and with buds infected with necrotic ring spot virus. Eighty-four per cent of 674 healthy buds and 72.3 per cent of 130 ring spot infected buds grew satisfactorily.

There seems to be sufficient evidence of deleterious effects of necrotic ring spot to be concerned about the control of this disease both in the nursery and in the orchard. Efforts for control have been directed primarily towards obtaining necrotic ring spot free propagative materials for production of necrotic ring spot free trees in the nurseries. Of perhaps greater interest to orchard operators is the fact

that a program directed towards producing necrotic ring spot free trees assures that the trees will also be free of cherry yellows, since yellows infected trees have always been found to contain necrotic ring spot virus. Cherry yellows is a devastating disease which often reduces yields 50 per cent or more. Detection of necrotic ring spot and yellows infected trees cannot be reliably accomplished by observation of suspects, for necrotic ring spot symptoms may not show after the initial infection, and yellows symptoms are not expressed if the temperature is much above 16° C. during the 30-day period after petal fall (Mills, 1946; Moore and Keitt, 1946). Yellows symptoms have not been observed on cherry trees in the scion orchards and the nursery blocks of southwest Iowa nurseries. Thus index plants are required for virus detection. Keitt and Clayton (1939) used Montmorency cherry trees as test plants in the original work which demonstrated that cherry yellows is of virus origin. Moore and Keitt (1949) reported on two indexing methods with Montmorency as the index plant. In their "direct" method buds from a suspect were inserted into virus free Montmorency trees. Symptom expression by the budded tree indicated that the suspect contained virus. In their "indirect" method, suspect trees were potted and forced in the greenhouse, then at bud break were inoculated with necrotic ring spot infected buds. If plants failed to develop symptoms they were assumed to have contained virus

which cross protected against virus inoculum. If symptoms developed the trees were assumed to have been virus free and budwood cut from them prior to inoculation was used for propagation.

Potentially there are a number of plants which could be used to index cherry trees, since Gilmer (1955) has shown that 122 Prunus species can be experimentally infected with necrotic ring spot virus. Hildebrand (1942b) reported the value of using peach trees for detection of yellows. Infected peach seedlings produced distinctive symptoms of rosette within three weeks from budding. Berkeley (1947) showed that peaches reacted to necrotic ring spot virus by undergoing shock symptoms of delayed foliation, die-back, and bark necrosis, followed by recovery if initial shock was not too severe. Seedlings inoculated with a yellows infected bud reacted similarly but in addition rosetted shoots persisted; thus, yellows can be diagnosed on this host.

Milbrath and Zeller (1945) demonstrated the value of the Kwanzan and Shirofugen varieties of Prunus serrulata for detection of latent viruses in stone fruits. Kwanzan trees inoculated in August develop in the following spring small curled leaves with split necrotic veins, which leaves are in tight rosettes with very little or no stem elongation. Small trees may be killed, or may produce new growth only below point of bud insertion. Shirofugen trees inoculated with

infected buds produce a local reaction with tissues in vicinity of the bud becoming necrotic and a mass of black gum forming in the region around the bud.

Fink (1950) indicated that Prunus tomentosa had promise as an index plant for necrotic ring spot virus. He found that P. tomentosa seedlings developed characteristic symptoms when inoculated either in the greenhouse or in the field with necrotic ring spot virus. Comparative studies in the field with peach seedlings revealed the detection of more infected plants with P. tomentosa than with peach. Young (1951) inoculated one-year-old P. tomentosa plants on peach roots with necrotic ring spot and with both necrotic ring spot and yellows. He reported that characteristic symptoms appeared on the plants inoculated with the source of necrotic ring spot alone, but not on plants inoculated with both necrotic ring spot and yellows. Thus he indicated that use of P. tomentosa would be restricted to detection of necrotic ring spot virus alone. However, Fink (1955) has shown that P. tomentosa seedlings will reliably express symptoms if inoculated with buds infected with both necrotic ring spot and yellows. He inoculated P. tomentosa seedlings and Montmorency trees with 19 sources of necrotic spot several of which were yellows infected and found that P. tomentosa expressed diagnostic symptoms as reliably as Montmorency. Fink indicated that this plant should have value as an index plant for in addition

to reacting characteristically with necrotic ring spot virus, the plants can be propagated from seed, they are available and can be used as seedlings and they are winter hardy.

The possibility of using herbaceous plants to index cherry trees became apparent after Moore, Boyle, and Keitt (1948) succeeded in infecting cucumbers by mechanical inoculation using the sap of infected cherry leaves. Boyle, Moore, and Keitt (1954) after a study of virus transmission to cucumbers from cherry trees known to be infected with different virus combinations, indicated a possible value of cucumber as an index plant.

Gilmer and Brase (1956) made a comparative study of the indexing value of cucumber and six Prunus species. They used 126 isolates to inoculate peach, Montmorency cherry, P. tomentosa, Albion and Shiro plums, and cucumber in the greenhouse, and Shirofugen in the field. Cucumber plants gave positive reactions to all isolates; Montmorency, Shirofugen, and Albion plum gave essentially equivalent numbers of positive reactions, with Shiro plum and P. tomentosa slightly less efficient. Peach seedlings were decidedly inferior. The authors pointed out that cucumber though an efficient plant for detection of virus in sour cherry trees, would not be reliable in detection of viruses in all Prunus species. They were unable to transmit virus from infected leaves of P. tomentosa seedlings to cucumber in a total of 52 trials.

The possible use of chemical tests for the detection of viruses in trees has been explored by Lindner (1948). He found that virus infected cherry leaves produced a brilliant red color after being heated in the presence of a reagent containing sodium hydroxide, cupric sulfate, sodium citrate, and water. He used a colorimeter to quantify the color observations and was thus able in preliminary tests on peach and sweet cherry to separate virus diseases into tentative groups. Lindner, Weeks, and Kirkpatrick (1951) examined several tissues for sensitivity to the chemical tests and found that leaf tissue was superior. They reported that the limitation to this method of virus detection was the inability to determine which viruses were present in infected tissue. Results from over 70,000 tests gave indication to the authors that few if any cherry or peach trees are free of virus. In further studies, Lindner, Kirkpatrick, and Weeks (1952) found that the products from acid hydrolysis of leaf tissue produced ultraviolet absorption spectra that appeared to be characteristic of virus diseases studied. Tomlinson and Woodbridge (1955) using the above method were unable to distinguish cherry trees known to be virus infected from trees which by other indexing methods had been shown to be virus free.

Early studies with necrotic ring spot infected trees revealed that symptoms are frequently not expressed each year (Berkeley et al., 1951). Milbrath (1952) examined 2,000

cherry trees for virus symptoms. He found 572 trees which did not express virus symptoms; however, when these trees were indexed on Shirofugen and Kwanzan 84.6 per cent were shown to be virus infected.

Moore (1945) has observed that necrotic ring spot virus latent in one strain of Montmorency may not be latent in another strain. He transmitted virus from an infected tree not expressing symptoms to a Montmorency tree which subsequently developed shock symptoms, but did not develop other symptoms thereafter. When he transmitted virus from this same source to another strain of Montmorency symptoms developed in the inoculated tree each year after the initial inoculation.

Moore and Keitt (1949) demonstrated that latent necrotic ring spot virus in sour cherry cross protects against necrotic ring spot virus transmitted to infected trees through grafted tissue. They made use of this cross protection in their aforementioned "indirect" method of indexing. Not all sources of necrotic ring spot are latent in sour cherry, as some sources carry viruses which produce characteristic necrotic ring spot symptoms each year. All sources known to produce recurrent necrotic ring spot in sour cherry are infected with yellows (Boyle, Moore, and Keitt, 1954).

Moore and Slack (1952) inoculated Montmorency cherry trees containing recurrent necrotic ring spot and yellows viruses with buds from four cherry trees known to carry

necrotic ring spot, yellows and prune dwarf viruses. Trees inoculated with three of the cherry sources exhibited shock symptoms on young leaves and subsequent leaves were symptomless; trees inoculated with the other source did not exhibit shock symptoms. When buds from the inoculated trees were placed on healthy Montmorency cherry trees no recurrent necrotic ring spot symptoms were expressed.

Cochran (1952) on the basis of experiments involving reinoculation of peach trees which contained "mild forms" of ring spot virus with "severe forms" concluded that ring spot forms in peach afford varying degrees of protection. Symptoms were not expressed on those reinoculated trees which had expressed both leaf symptoms and bark necrosis. The mild forms afforded protection against one but were severely shocked by the other two severe forms. Symptoms were less severe than on trees which had not been infected with mild forms.

A study of latency of necrotic ring spot virus in P. mahaleb was made by Hobart and Buchholtz (1955) for the purpose of determining if the "indirect" method of indexing, which requires distinct latency, could be used to detect virus present in lots of P. mahaleb seedlings used for understocks in cherry production. They inoculated P. mahaleb seedlings with each of 14 sources of necrotic ring spot virus and the following year reinoculated part of these seedlings. They found that symptoms were expressed by the plants whether they were reinoculated or not; thus necrotic

ring spot virus is not latent in P. mahaleb and the "indirect" method of indexing could not be used.

Fridlund (1954a) inoculated P. tomentosa seedlings with five sources of necrotic ring spot, then several months after the acute symptom phase pruned and completely defoliated the trees. Some of these trees were reinoculated with one of seven virus sources. He found only one of 13 combinations which produced acute symptoms in the reinoculated plants and these symptoms were not as severe as those in the inoculated healthy control plants; thus some interference with symptom development had occurred.

The spread of necrotic ring spot virus has been studied by several investigators and some analyses made concerning rate of spread. Willison, Berkeley, and Chamberlain (1948) reported the results of yearly surveys for presence of necrotic ring spot in several cherry orchards. They considered trees which had only mild symptoms as having been infected prior to survey and those with severe or moderate symptoms as representing new infections. Their analysis indicated that on the average 13.2 to 19.0 per cent of trees considered healthy became infected each year, and that the rate of spread of necrotic ring spot was correlated negatively with prevalence of infective sources. This unexpected correlation was attributed to having considered trees as healthy when actually they were infected with virus which was latent.

Hildebrand (1953) reported on a typical result from orchard surveys. All but 12 trees of 207 were observed to express yellows symptoms, and these 12 when indexed were shown to contain necrotic ring spot virus. He also reported a survey of a young Montmorency orchard of 396 trees, which showed a total of 2 trees infected with necrotic ring spot virus in 1941, 8 in 1942, 36 in 1943, and 105 in 1944. Willison (1949) reported that on trees (892) in five orchards examined for the first time there were shock symptoms on only two.

The rate of spread of necrotic ring spot virus is admittedly not clear as most of the evidence for spread has come from surveys which did not involve indexing, so that latent infections could not be determined. The presence of shock symptoms has been taken as evidence of new infection in most of the surveys; however, the fact that a tree may express shock symptoms on some branches one year and on other branches another year makes use of this criterion unreliable in determination of new infections.

A few studies of spread have been made in which trees studied were indexed. Fridlund (1954a) investigated the spread of necrotic ring spot virus in two, five-year-old plum orchards which in all contained 273 trees, 50 of which were of a variety which is universally contaminated with necrotic ring spot virus. He indexed all trees and found the 50 trees

of the contaminated variety to be infected, and only two others.

Hobart, Fink, and Buchholtz (1955) reported evidence of spread of necrotic ring spot virus in sour cherry blocks in southwest Iowa nurseries. Their studies involved indexing 100-tree samples in nursery blocks, and later in the same or another season indexing the same or another sample. In all tests they found more trees infected on the second indexing than on the first. In three instances samples in unisolated blocks had relatively greater percentage increases of infected trees (28, 9, 11) than in isolated blocks (2, 6, 4).

Hobart (1956) explored the possibility that the spread in nursery rows might be due to transmission through root grafts. Experimentally he was able to transmit necrotic ring spot virus from one Prunus species to another through root grafts, however from examination of roots of plants in the nursery rows he concluded that this was not a significant means by which virus is spread in the nursery.

To date there is little evidence which indicates how necrotic ring spot virus is transmitted naturally. Keitt and Clayton (1943) had some suggestion that leaf hoppers transmitted necrotic ring spot virus, however this has not been substantiated and no further evidence on transmission by insects has been forthcoming.

Much of the necrotic ring spot virus present in nurseries and orchards is attributable to virus present in rootstocks

which are used for cherry production, Prunus mahaleb and P. avium var. Mazzard. Cochran (1946) demonstrated the passage of necrotic ring spot virus through seeds of P. avium var. Mazzard and Cation (1949) through seed of P. mahaleb. Other Prunus species in which seed transmission has been reported are: peach (Cochran, 1950), Italian prune (Ehlers, 1957), Stockton Morello (Nyland, 1952) and P. americana (Hobart, 1954).

Experimentally necrotic ring spot virus has been transmitted by the usual grafting methods from Prunus to Prunus. Moore, Boyle, and Keitt (1948) mechanically transmitted necrotic ring spot virus from Montmorency to cucumber by grinding young leaves and inoculating cotyledons with the undiluted sap. They were able to transmit the virus from cucumber to cherry by placing infected cucumber leaves under the bark of cherry trees. Hobbs (1951) in studies of susceptibility of a number of cucurbits found that 46 of 47 cucumber varieties and 4 of 9 pumpkin varieties were susceptible, but none of 12 squash or 7 watermelon were susceptible. All attempts by Hobbs to reinfect cherry with virus from cucumber by patch grafting and mechanical techniques failed.

Heinis (1956) mechanically transmitted virus to cucumber from 20 of 23 trees of which 22 were infected with ring spot virus. Comparisons were made between the severity of

reactions on cucumber and the severity of reactions on Prunus hosts. The comparisons suggested that the virus transmitted to cucumber was the ring spot virus.

Milbrath (1953) transmitted a virus from cherry trees to cucumber using sap prepared from flower petals. McWhorter (1953) found that inocula prepared from cherry flowers superior to those prepared from leaves. Heinis (1956) in comparisons of the effectiveness of cherry leaves and cherry flowers as inocula, found leaves to be more effective. Ehlers and Moore (1957) have succeeded in mechanically transmitting virus to herbaceous plants from the pollen of infected cherry trees.

Fulton (1957) has transmitted Prunus virus from herbaceous plants to some Prunus seedlings. He found P. pennsylvanica and P. mahaleb were more readily infected (33-29 per cent) than were P. cerasus, P. virginiana or P. serotina (10-5 per cent). After seedlings developed about eight leaves, they were relatively insusceptible. No infection resulted when P. tomentosa or P. persica seedlings were inoculated.

MATERIALS AND METHODS

Virus Sources

Eighteen sources of necrotic ring spot virus were used, 17 supplied by Dr. J. D. Moore, University of Wisconsin, and 1 (Fruitmorency) supplied by Shenandoah (Lake's) Nurseries. The latter source could be obtained in large quantity, thus was used as inoculum in most of the transmission experiments. The description of these sources is contained in Table 1. Gilbert Montmorency budwood free of necrotic ring spot virus was supplied by Inter-State Nurseries. All budwood was stored in moist sphagnum moss at a temperature of 33° C.

Trees

Prunus tomentosa Thunb. seedlings were obtained from Plumfield Nurseries, Fremont, Nebraska. The trees from which seed was taken to grow these seedlings had previously been indexed and found to be free of necrotic ring spot virus.

Prunus cerasus variety Gilbert Montmorency trees were furnished by Inter-State Nurseries, Hamburg, Iowa. They were tested and found to be free of necrotic ring spot virus prior to their use in experiments.

Prunus cerasus "variety" Fruitmorency trees were supplied by Shenandoah (Lake's) Nurseries, Shenandoah, Iowa. The exact

Table 1. Description of virus sources

Virus source[a]	Description[b]
B-1-12	necrotic ring spot alone
G-2-1	necrotic ring spot alone
G-5-1	necrotic ring spot alone
M-3-17	necrotic ring spot and yellows
M-6-19	necrotic ring spot (recurrent) and yellows
M-6-30	necrotic ring spot (recurrent) and yellows
S-1649	necrotic ring spot (recurrent) and yellows
S-1563	necrotic ring spot (recurrent) and yellows
B-3-23	necrotic ring spot, yellows and prune dwarf
M-7-74	necrotic ring spot, yellows and prune dwarf
B-3-22	necrotic ring spot, yellows and prune dwarf
G-2-7	necrotic ring spot, yellows and prune dwarf
G-20-5	necrotic ring spot, yellows and prune dwarf
S-5105	necrotic ring spot, yellows and prune dwarf
HSB-15-7	necrotic ring spot, yellows and prune dwarf
B-1-35	necrotic ring spot, yellows, prune dwarf and green ring yellows
M-5-74	necrotic ring spot and green ring yellows
Fruitmorency	necrotic ring spot

[a]Sources of virus except for Fruitmorency furnished by Dr. J. D. Moore.

[b]Description is with reference to sour cherry and Italian prune trees.

origin of this variety is not known. It was obtained by Shenandoah Nurseries from Fruitland Nursery, Fruitland, Idaho, but this nursery does not have a record of its origin. All trees of this variety that have been tested contain necrotic ring spot virus.

Prunus serrulata Lindl. variety Shirofugen trees growing on the premises of Inter-State Nurseries were made available for summer indexing.

Handling of Trees

All trees used in the greenhouse were grown in unsteamed compost soil in clay pots. The P. tomentosa seedlings were pruned to a height of about 18 inches. Branches were pruned so that only a whip remained. Some pruning of roots was necessary to enable placement in five-inch pots. The branches and root system of Gilbert Montmorency and Fruitmorency trees were pruned prior to placing trees in eight-inch pots. These trees were placed on greenhouse benches immediately after potting. The P. tomentosa seedlings after potting were either placed on greenhouse benches for immediate use or stored out of doors until needed. The night temperatures of the greenhouse rooms used ranged from 21 to 24° C.

Plants which were retained for another greenhouse season were removed to a field plot. The trees were left in pots which were placed in the ground to a depth sufficient to cover

them. In late October these pots were removed from the field and similarly placed in the ground in a site near the greenhouse. They were usually brought into the greenhouse during January, which assured their having received sufficient exposure to cold for the breaking of dormancy.

Some P. tomentosa seedlings were grown in the greenhouse in the winter and then kept over summer in the field so that budwood could be collected from them in the fall. Such seedlings were removed from pots and lined out in the field plot. Budwood was collected from these seedlings in late October and stored as indicated above.

Inoculations

Inoculations of seedlings were made about three inches above the soil line. The Gilbert Montmorency trees were inoculated about three inches above the stock-scion union. The inoculations with buds were made using the chip-bud method described by Moore (1945), which involved removing a shield of bark about three-fourths inch in length from the stem of tree being inoculated, and replacing with a bud borne on tissue the same length as the chip removed. The shield was wrapped with a standard budding rubber in such a way that the bud was not covered. Inoculations in which bark was used as inoculum were made in essentially the same way. Bud and bark inocula were not wooded prior to use. When leaf tissues were used as

inoculum, a bark flap was cut in the stem of the seedlings, the tissues placed under the bark flap, and the flap wrapped with a budding rubber. To remove inoculum the budding rubber was cut, then the inoculum tissue lifted with a knife and pulled off. The tissues in the vicinity of the inoculum were scraped with a knife to assure that none of the inoculum remained.

Inoculations with sap were made by placing a piece of sterile cotton which had been soaked in sap under a flap of bark and then wrapping the flap with a budding rubber. Crude sap was prepared by grinding young leaves which were showing symptoms in a mortar with a small quantity of distilled water. Sap free of leaf tissue was prepared as above, then centrifuged at 5,000 RPM for five minutes. The supernatant was used as inoculum.

Root grafts of seedlings were made using approach grafts, which were wrapped with budding rubbers. The plant pairs were placed in five-inch pots. In experiments involving contact of injured stems and roots, the tissue of a given pair of seedlings was broken by scraping with a knife. The plants were held together at the point of injury. In the stem contact experiments the plants of a pair were grown in separate pots.

EXPERIMENTS

Latency

Some sources of necrotic ring spot contain virus which is latent in sour cherry trees. Infection of sour cherry trees with these viruses usually results in symptoms being expressed the year of infection but not subsequently. Moore and Keitt (1949) made use of this fact in developing a method for detection of necrotic ring spot virus in sour cherry trees. Trees to be tested were inoculated with a bud containing necrotic ring spot virus. If symptoms developed, the tree was considered to have been necrotic ring spot virus free and budwood collected from it prior to inoculation was used for propagation. If symptoms were not expressed due to cross protection phenomena the tree was considered to have contained virus. This method of indexing is not generally used for indexing "seed" and scion trees as it involves infection of these trees. Most of the indexing of cherry trees is accomplished by placing buds of suspects on healthy index plants with symptom expression indicating that suspects contain virus. It is obvious that if the index plant contained latent virus erroneous results would be obtained. Thus it is desirable to know if necrotic ring spot virus is latent in index plants.

P. tomentosa, since its introduction by Fink in 1950, has been gaining favor as a reliable index plant for detection of necrotic ring spot virus in Prunus species. To determine if necrotic ring spot virus is latent in this plant, seedlings were inoculated with necrotic ring spot virus in 1957 and one year later observed for necrotic ring spot symptoms after some of the infected seedlings had been reinoculated.

Another latency experiment involved a study of symptom expression on new foliage of infected plants that had been cut back and defoliated.

Symptom expression of necrotic ring spot virus infected P. tomentosa seedlings one year after inoculation

Each of 18 sources of necrotic ring spot virus was inoculated into ten healthy P. tomentosa seedlings at bud break. Twenty plants were held as checks, ten of which were not inoculated and ten of which were budded with virus-free Gilbert Montmorency buds. All trees inoculated expressed necrotic ring spot symptoms and these along with checks were placed in a field plot over summer. The trees were heeled in near the greenhouse in the fall and brought into the greenhouse in January 1958. Eighteen of the 200 plants did not survive over summer. To determine if virus was still present in inoculated trees, all surviving plants were indexed on P. tomentosa seedlings, with the exception of 18 whose stems had died back

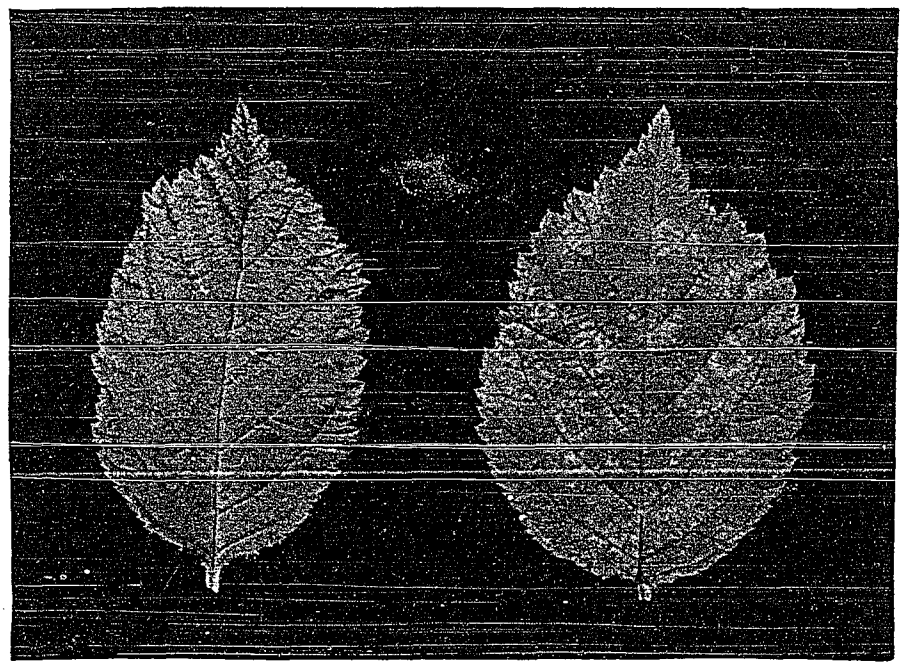
so that budwood could not be collected from them. All of the inoculated plants which were indexed gave necrotic ring spot positive reactions. None of the checks gave positive reactions.

Six of the ten plants originally infected with a given source were reinoculated, two with the same source, two with a similar source, and two with a dissimilar source. The other four were not reinoculated. A source was considered similar if it contained the virus components of the original source, and dissimilar if it either contained some component not in the original or lacked some component in the original source. Some field losses prevented strict adherence to this reinoculation plan.

The plants were observed for expression of symptoms for four months after reinoculation. Symptoms were classified into three groups: mild, moderate (Figure 1), and severe (Figure 2). Symptoms were classified as mild if leaf mottling required close comparison with checks for detection, as moderate if leaf mottling was readily apparent but not accompanied by necrosis, and as severe if leaves in addition to mottle had some necrotic tissue. The symptom expression of reinoculated and non-reinoculated plants is shown in Table 2. It is seen that many trees did not re-express symptoms, that most trees re-expressing had mild symptoms, and that necrotic symptoms were seldom observed.

Figure 1. *P. tomentosa* leaves. Left: plant not inoculated. Right: plant inoculated in 1957 with necrotic ring spot virus and reinoculated in 1958 (moderate reaction)

Figure 2. *P. tomentosa* leaves. Left: plant not inoculated. Right: plant inoculated in 1957 with necrotic ring spot virus and reinoculated in 1958 (severe reaction)



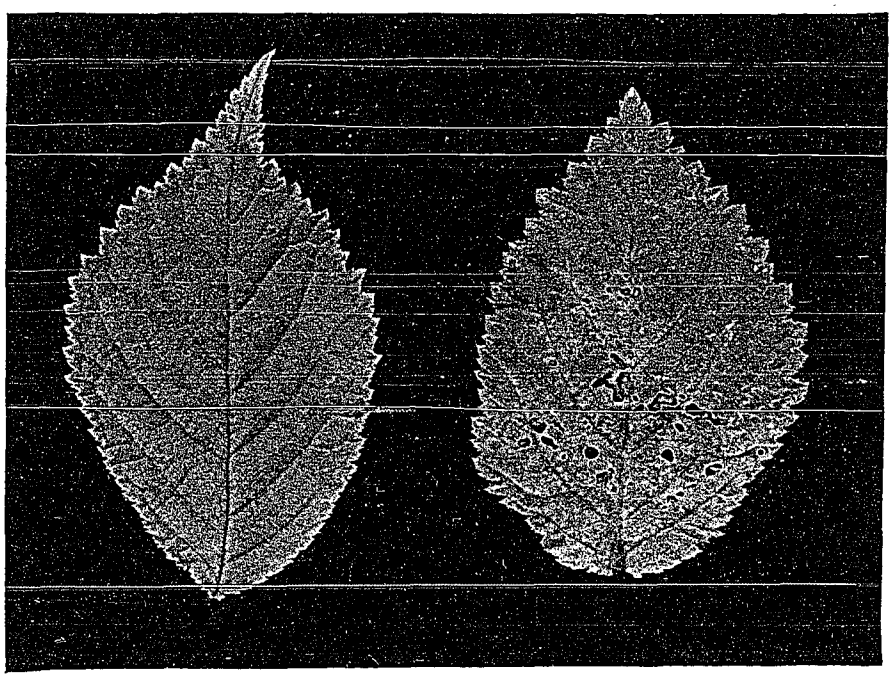


Table 2. Symptom expression in 1958 by P. tomentosa seedlings some of which had been inoculated with necrotic ring spot virus only in 1957, some reinoculated in 1958. Greenhouse

Source and year of inoculation		Number of seedling trees inoculated	Symptom expression			
1957	1958		None	Mild	Moderate	Severe
B-1-12	None	2	1		1	
B-1-12	B-1-12	2	2			
B-1-12	G-5-1	2	1			1
B-1-12	B-3-22	1			1	
G-2-1	None	4		4		
G-2-1	G-2-1	2	1	1		
G-2-1	G-5-1	2		2		
G-2-1	M-7-74	2			2	
G-5-1	None	4			1	3
G-5-1	G-5-1	2		2		
G-5-1	B-1-12	2		2		
G-5-1	M-6-19	2		1	1	
M-3-17	None	2	1	1		
M-3-17	M-3-17	2		2		
M-3-17	B-3-23	1		1		
M-3-17	B-1-12	2			2	
M-6-19	None	4	1	2	1	
M-6-19	M-6-19	2		2		
M-6-19	B-3-23	2	1	1		
M-6-19	G-5-1	1	1			
M-6-30	None	4		1	1	2
M-6-30	M-6-30	2			2	
M-6-30	M-6-19	2			2	
M-6-30	M-7-74	1		1		
S-1649	None	3		1		2
S-1649	S-1649	2			2	
S-1649	G-2-1	2			2	
S-1649	M-7-74	2			2	
S-1563	None	3		2	1	
S-1563	S-1563	2			2	
S-1563	G-5-1	2		1		1
S-1563	M-6-19	1				1

Table 2 (continued)

Source and year of inoculation 1957	1958	Number of seedling trees inoculated	Symptom expression None	Mild	Moderate	Severe
B-3-23	None	4	1	3		
B-3-23	B-3-23	2	2			
B-3-23	M-3-17	2		2		
B-3-23	G-2-7	2	2			
M-7-74	None	4		4		
M-7-74	M-7-74	2	2			
M-7-74	M-3-17	2	2			
M-7-74	B-1-12	2		1	1	
B-3-22	None	3		2		1
B-3-22	B-3-22	2	2			
B-3-22	G-2-7	1		1		
B-3-22	M-7-74	2	1	1		
G-2-7	None	4	4			
G-2-7	G-2-7	2	2			
G-2-7	G-20-5	2	2			
G-2-7	G-5-1	2	1	1		
G-20-5	None	4	4			
G-20-5	G-20-5	2	2			
G-20-5	B-3-22	2	1		1	
G-20-5	M-3-17	2	2			
S-5105	None	3	2			1
S-5105	S-5105	2		1	1	
S-5105	B-3-22	2		2		
S-5105	M-3-17	1	1			
HSB-15-7	None	3		2	1	
HSB-15-7	HSB-15-7	2		2		
HSB-15-7	G-20-5	2		2		
HSB-15-7	B-3-23	2		2		
B-1-35	None	4		4		
B-1-35	B-1-35	2	1	1		
B-1-35	B-1-12	2		2		
B-1-35	B-3-22	2			2	

Table 2 (continued)

Source and year of inoculation		Number of seedling trees inoculated	Symptom expression			
1957	1958		None	Mild	Moderate	Severe
M-5-74	None	4	3		1	
M-5-74	M-5-74	1			1	
M-5-74	G-20-5	2		2		
M-5-74	G-5-1	2		2		
Fruit-morency	None	3		2	1	
Fruit-morency	Fruit-morency	2		2		
Fruit-morency	M-6-19	2		2		
Fruit-morency	B-1-12	2		2		
None	None	4	4			
None	B-1-12	2				2
None	M-3-17	2				2
None	G-20-5	2				2
None	None	10	10			

Plants originally inoculated with sources which contained virus recurrent in sour cherry (M-6-19, M-6-30, S-1649, S-1563) had more moderate and severe class symptoms than plants inoculated with nonrecurrent sources, with the exception of plants inoculated with source M-6-19. Only one plant in nine inoculated with this source had symptoms of a class more prominent than mild. Whether infected plants were reinoculated or not seemed to have little influence on expression of symptoms.

Symptom expression of necrotic ring spot virus infected P. tomentosa seedlings after defoliation

A study was made of symptom expression on new foliage of infected seedlings which had been cut back and completely defoliated. Trees which had been used to index the seedlings in the experiment reported in Table 2, and which had expressed symptoms of necrotic ring spot were cut back and defoliated. Thirty-six plants that had not been infected were also treated in the same way.

Approximately one-half of the infected trees were re-inoculated with either Fruitmorency buds or buds from source M-6-30 (recurrent) with the exception of plants previously inoculated with source M-6-30, none of which were reinoculated. Twenty plants previously not infected were inoculated, ten with Fruitmorency buds and ten with buds of source M-6-30. Inoculations were made four or five days after defoliation. Symptom observations were made for six weeks after inoculation and the symptoms classified as in the previous experiment.

Table 3 shows the expression of symptoms on the defoliated plants. The only trees showing severe symptoms were those originally virus-free check plants that had been inoculated. Trees originally inoculated with the sources (recurrent) S-1649 and S-1563 were more frequently found with moderate class symptoms than trees inoculated with other sources,

Table 3. Symptom expression of previously infected P. tomentosa seedlings after defoliation and re-inoculation. Greenhouse, 1958

Source and month of inoculation		Number of seedling trees	Symptom expression			
March	April	inoculated	None	Mild	Moderate	Severe
B-1-12	None	4	4			
B-1-12	FM[a]	6	6			
G-2-1	None	8	4	4		
G-2-1	FM	10	5	5		
G-5-1	None	10	1	9		
G-5-1	M-6-30	10	1	8	1	
M-3-17	None	4	2	2		
M-3-17	FM	6	2	3	1	
M-6-19	None	8	4	4		
M-6-19	FM	8	6	2		
M-6-30	None	14	9	4	1	
S-1649	None	7		4	3	
S-1649	FM	6			6	
S-1563	None	8		4	4	
S-1563	FM	8		7	1	
B-3-23	None	6	4	2		
B-3-23	FM	10	9	1		
M-7-74	None	8	7	1		
M-7-74	FM	8	5	3		
B-3-22	None	6	5	1		
B-3-22	FM	4	1	3		
G-2-7	None	10	9	1		
G-2-7	FM	10	10			
G-20-5	None	8	8			
G-20-5	FM	8	8			

[a]FM = Fruitmorency.

Table 3 (continued)

Source and month of inoculation		Number of seedling trees inoculated	Symptom expression			
March	April		None	Mild	Moderate	Severe
S-5105	None	6	4	2		
S-5105	FM	6	6			
HSB-15-7	None	6	4	1	1	
HSB-15-7	FM	6	2	4		
B-1-35	None	10	3	5	2	
B-1-35	M-6-30	10	7	3		
M-5-74	None	7	5	1	1	
M-5-74	FM	10	5	1	4	
FM	None	4	2	2		
FM	FM	6	3	3		
None	M-6-30	10		1	1	8
None	FM	10		3	5	2
None	None	16	16			

except those trees inoculated with source M-5-74. The seedlings inoculated with source M-6-30 (recurrent) which were not reinoculated, did not prominently express symptoms. Trees inoculated originally with recurrent source M-6-19 did not express any moderate or severe symptoms.

Transmission

The means by which necrotic ring spot virus is naturally transmitted is not known. Spread of virus in established

orchards has been reported by a number of investigators who have made yearly surveys of cherry orchards. The latent and recurrent nature of necrotic ring spot virus make these surveys, which did not involve indexing, unreliable in estimating amount of spread. Fridlund (1954) in a survey of two, five-year-old plum orchards found through indexing that of 223 trees in close proximity to 50 trees of a universally contaminated variety, Mt. Royal, only two contained necrotic ring spot virus. Hobart, Fink, and Buchholtz (1955) in studies of virus spread in southwest Iowa nurseries found through experiments involving indexing an indication of virus spread in blocks of nursery rows. The low incidence of spread suggested a possible mechanical transmission; thus experiments were designed to examine this possibility.

The major experiments on transmission reported here involved determining the length of time that infected buds and bark must be in contact with cherry trees if symptoms of necrotic ring spot are to develop. Other experiments involved determination of whether virus was transmitted from diseased to healthy plants when roots or stems were in contact. Some preliminary studies were made using leaf and sap inocula for transmission of virus to P. tomentosa.

Contact periods required for the transmission of necrotic ring spot virus

Tissue containing necrotic ring spot virus was placed on

P. tomentosa seedlings and on P. cerasus variety Gilbert Montmorency trees for varying periods of time and determinations made of the length of time inoculum had to be in contact with the trees for symptoms ultimately to develop.

P. tomentosa seedlings inoculated with P. cerasus variety Fruitmorency tissue. P. tomentosa seedlings were inoculated when dormant, at bud break, and when the buds were open (Figure 3) with dormant buds, open buds, and dormant bark. Preliminary experiments had shown that symptoms appeared as soon in plants inoculated with one bud as with two, so in all experiments, at the time of inoculation only one bud (or other inoculum) was used. All inoculations were made with Fruitmorency tissue. Inoculum was left in contact with the seedlings for 1, 2, 4, 8, and 42 days. Final observations for symptoms of necrotic ring spot were made six weeks or more after inoculation. Plants not expressing symptoms were removed from the greenhouse and kept over summer in the field. Budwood was collected from these plants in the fall and tested for presence of virus on P. tomentosa seedlings. There was good conformity between presence of virus as determined by observation of the inoculated plants and presence of virus as determined by indexing. The number of plants to which virus was transmitted is shown in Table 4.

A similar experiment was completed in 1958 using the same budwood and seedling conditions as in the first experiment,

Figure 3. P. tomentosa seedlings. Left to right: dormant, at bud break, and with buds open

Table 4. Transmission of necrotic ring spot virus to P. tomentosa seedlings after contact with infected P. cerasus variety Fruitmorency buds and bark for 1, 2, 4, 8, and 42 days. Greenhouse, 1957

Seedlings inoculated with:	Contact period (days)				
	1	2	4	8	42
Seedlings inoculated when dormant					
Buds, dormant	0[a]	0	0	10	10
Buds, open	0	0	1	10	10
Bark, dormant	0	0	0	9	10
Seedlings inoculated at bud break					
Buds, dormant	0	0	1	10	10
Buds, open	0	0	1	10	10
Bark, dormant	0	0	1	10	10
Seedlings inoculated when buds 1/2" open					
Buds, dormant	0	0	10	10	10
Buds, open	0	0	5	10	10
Bark, dormant	0	0	5	10	10

[a]Each datum indicates the number of plants in 10 which expressed symptoms.

but with contact periods of 2, 3, 4, 6, and 8 days. The results are shown in Table 5.

The experiments reported in Tables 4 and 5 show that contact periods of three days or less were not sufficient to induce development of symptoms in the inoculated plants.

Table 5. Transmission of necrotic ring spot virus to P. tomentosa seedlings after contact with infected P. cerasus variety Fruitmorency buds and bark for 2, 3, 4, 6, and 8 days. Greenhouse, 1958

Seedlings inoculated with:	Contact periods (days)				
	2	3	4	6	8
Seedlings inoculated when dormant					
Buds, dormant	0[a]	0	0	0	3
Buds, open	0	0	0	1	9
Bark, dormant	0	0	0	1	6
Seedlings inoculated at bud break					
Buds, dormant	0	0	0	9	9
Buds, open	0	0	0	9	8
Bark, dormant	0	0	1	9	10
Seedlings inoculated when buds 1/2" open					
Buds, dormant	0	0	3	10	10
Buds, open	0	0	0	6	9
Bark, dormant	0	0	0	9	10

[a]Each datum indicates the number of plants in 10 which expressed symptoms.

Contact periods required for transmission were less for seedlings with buds open than with seedlings at bud break, and less for seedlings at bud break than with dormant seedlings. Transmission was seemingly most rapid when dormant buds were inoculated into seedlings which had buds open.

P. tomentosa seedlings inoculated with P. tomentosa tissue. The inoculum tissue in the previous experiments was of a different species (*P. cerasus*) than the tissue to which it was grafted (*P. tomentosa*). To determine the contact period required for transmission when host and inoculum tissue were of the same species, *P. tomentosa* seedlings at bud break were inoculated with buds from *P. tomentosa* seedlings which previously had been inoculated with necrotic ring spot virus and which had subsequently shown symptoms. The contact periods were 1, 2, 3, 4, 6, 8, and 42 days. The number of trees to which virus was transmitted is shown in Table 6. The results are similar to those with plants under comparable conditions (seedlings at bud break; inoculum, buds open) in the experiment recorded in Table 5.

P. cerasus variety Gilbert Montmorency inoculated with P. cerasus variety Fruitmorency tissue. To determine if similar contact periods were required for transmission of necrotic ring spot virus to sour cherry trees (*P. cerasus*) as to *P. tomentosa* seedlings, 50 Gilbert Montmorency trees were inoculated at bud break with dormant buds of Fruitmorency. Contact periods were 1, 2, 4, 8, and 42 days. The results are recorded in Table 7, and are shown to be similar to the results under comparable conditions recorded in Table 4.

To determine if transmission might have occurred with a contact period of six days, nine of the ten trees on which

Table 6. Transmission of necrotic ring spot virus to *P. tomentosa* seedlings after contact with infected *P. tomentosa* buds for 1, 2, 3, 4, 6, 8, and 42 days. Greenhouse, 1958

P. tomentosa seedlings inoculated at bud break with:	Contact period (days)						
	1	2	3	4	6	8	42
P. tomentosa buds	0[a]	0	0	0	8	7	10

[a]Each datum indicates the number of plants in 10 which expressed symptoms.

Table 7. Transmission of necrotic ring spot virus to *P. cerasus* variety Gilbert Montmorency trees after contact with infected *P. cerasus* variety Fruitmorency buds for 1, 2, 4, 8, and 42 days. Greenhouse, 1958

Gilbert Montmorency trees inoculated at bud break with:	Contact period (days)				
	1	2	4	8	42
Fruitmorency buds	0[a]	0	1	9	10

[a]Each datum indicates the number of plants in 10 which expressed symptoms.

inoculum had remained only one day were pruned back so no foliage remained. Five days after cutting back, they were inoculated with Fruitmorency buds. These buds were removed at the end of six days. Five of the nine trees expressed necrotic ring spot symptoms thus indicating transmission had occurred with contact periods comparable to P. tomentosa under similar conditions (Table 5, seedlings at bud break). Those trees on which inoculum was left for four and eight days and which did not express symptoms were indexed on P. tomentosa seedlings and found to be necrotic ring spot negative.

Transmission of necrotic ring spot virus from diseased to healthy plants through contact of roots and stems

Experiments were designed to determine whether virus is transmitted from infected to healthy P. tomentosa seedlings when roots or stems are in contact.

Root contact. Hobart (1956) has shown that transmission of necrotic ring spot could occur through root grafts of cherry trees, but from nursery observations he felt this method was not significant. Close association of roots of P. tomentosa seedlings placed more than one to a pot for routine indexing affords opportunity for contact of roots during the six-week period plants are kept growing in greenhouse after being budded. A study was made to determine likelihood of transmission of virus from diseased to healthy

seedlings whose roots were in contact for a six-week period.

In a preliminary experiment bark shields from the roots of infected P. tomentosa seedlings were used to inoculate healthy P. tomentosa seedlings. All ten plants so inoculated expressed typical symptoms. Experiments were then designed so that roots of healthy and diseased plants were placed in varying degrees of contact. In the first experiment roots of pairs of healthy seedlings were tied together; then plant pairs were placed in five-inch pots. One plant of each pair was inoculated at the time of potting. None of the uninoculated trees in the ten pairs treated expressed symptoms.

In the second experiment the roots of two healthy plants were scraped with a knife so that tissues were broken; then the injured roots were tied together. One tree of each pair was inoculated. Of ten pairs, none expressed symptoms other than the inoculated seedlings.

In another root contact experiment, the roots of pairs of seedlings were approach grafted and wrapped with budding rubbers to make the grafts secure. Thirteen pairs of healthy seedlings were grafted. One plant of each of ten pairs was then inoculated, the other pairs serving as checks. Two uninoculated plants in contact with infected plants expressed typical necrotic ring spot symptoms. An examination of the root grafts six weeks after inoculation revealed very slight healing in of tissue, even in those pairs where transmission had occurred.

The root contact experiments indicate that there is not much likelihood of transmission of virus between P. tomentosa roots which are in superficial contact. Thus the placing of more than one seedling to a pot during routine indexing would not be objectionable from the standpoint of possible transmission of virus from the roots of one seedling to another.

Stem contact. The experiments reported in Tables 4 and 5 would indicate that there is little likelihood of transmission of necrotic ring spot virus from one plant to another when branches are in occasional contact. The following experiments were designed to determine if virus would be transmitted from diseased to healthy plants if stems were in superficial contact for periods of time greater than that necessary for transmission of virus through grafts. The stems of two healthy seedlings in separate pots were tied together. One member of the pair was inoculated with an infected bud. Of twenty pairs so treated and three pairs of checks, none contained uninoculated plants which expressed symptoms.

In a similar experiment, the stems of two plants were scraped with a knife and then tied together at the points of injury. Of twenty pairs treated and five pairs of checks, only one uninoculated plant expressed symptoms.

It was thought that transmission would be more likely to occur if instead of using plants inoculated at the time of contact, plants were used that previously had been

infected and shown symptoms. Accordingly the stems of healthy plants were placed in contact with stems of previously infected seedlings. All stems were injured by scraping with a knife prior to contact. Thirty pairs were treated in this way, with 10 being left in contact for 7 days, 10 in contact for 10 days, and 10 in contact for 42 days. Of the thirty pairs only one uninoculated plant (10 day contact period) expressed symptoms.

Though the data are too limited to be conclusive it would seem that stem contact would not be an important means by which virus is transmitted from one tree to another.

Reaction of P. tomentosa seedlings inoculated with leaf tissue and sap

Experimentally it has been difficult to infect Prunus leaves by direct application of virus-containing materials to the leaves. Fulton (1957) has succeeded in infecting young seedlings of some species of cherry by inoculating leaves with sap from the cotyledons of cucumber infected with virus from Prunus. Experiments were undertaken to determine if leaves and sap from infected Prunus could be used to infect P. tomentosa seedlings. However instead of attempting transmission to leaves, efforts were made to effect transmission to stem tissue.

Leaf tissue. Young P. tomentosa leaves showing symptoms of necrotic ring spot were macerated and then placed under

bark flaps of healthy P. tomentosa seedlings. Of ten plants treated none developed symptoms. In a similar experiment Fruitmorency leaves were used. Again none of the ten plants inoculated developed symptoms.

Nonmacerated leaf tissue in the form of leaf strips was next tried. Young Gilbert Montmorency leaves expressing symptoms were cut into strips and the strips placed under bark flaps of P. tomentosa stems. No transmission to the ten inoculated plants occurred. Next ten seedlings of P. tomentosa were inoculated with a single strip of Gilbert Montmorency leaf which was about the same width as the bark flap. One plant in ten expressed symptoms. In another test, several strips of P. tomentosa leaves were used as inoculum. Two of ten plants that were inoculated expressed symptoms.

Though transmission was not consistent, these experiments show that infected leaf tissue can be used to transmit necrotic ring spot virus to stem tissue of P. tomentosa seedlings.

Sap inoculum. P. tomentosa seedlings at bud break were inoculated with crude sap obtained from young leaves of necrotic ring spot infected Fruitmorency trees. Inoculations were made by placing sterile cotton which had been soaked in sap under bark flaps, then wrapping the flaps with budding rubbers. Eight of the ten plants so inoculated expressed typical necrotic ring spot symptoms. All ten plants were kept

over summer in the field and budwood taken from them in the fall. All ten trees were shown to contain necrotic ring spot virus when indexed on P. tomentosa. In a similar experiment ten seedlings were inoculated at the open bud stage with crude sap of Fruitmorency leaves. Six of the ten inoculated trees expressed symptoms. When these trees were indexed in the winter of 1958 all ten gave necrotic ring spot positive reactions on P. tomentosa.

Further experimentation with sap inocula in 1958 involved placing crude sap and sap free of leaf tissue (centrifuged) under bark flaps of P. tomentosa seedlings. Ten seedlings were inoculated at bud break with crude sap from infected Gilbert Montmorency leaves. None became infected. In another experiment seedlings were inoculated with Gilbert Montmorency leaf sap which was free of leaf tissue. None of the ten plants inoculated expressed symptoms. In a final experiment, 40 seedlings of P. tomentosa were inoculated at bud break by placing cotton soaked in crude sap from Gilbert Montmorency leaves under bark flaps. The cotton containing the sap inoculum was removed from 10 plants at 2, 4, and 8 days, and was not removed from 10 plants. None of the 40 inoculated plants developed symptoms.

These experiments show that it is possible to transmit necrotic ring spot virus from crude sap of infected cherry leaves to stem tissue of P. tomentosa seedlings.

Indexing

P. tomentosa seedlings have proven to be reliable index plants for detection of necrotic ring spot virus. The trees in the cherry scion orchards in southwest Iowa nurseries have been repeatedly indexed with this plant. To get a check on how effective this indexing with P. tomentosa has been, trees which had been indexed repeatedly on P. tomentosa were re-indexed on Shirofugen trees.

The usual method of indexing with P. tomentosa involves budding seedlings at bud break either in the greenhouse or in the field and observing newly formed foliage for development of symptoms. Very few infected trees are now found when orchard trees which have been indexed several times are re-indexed. Thus most of the index plants used do not become infected. An experiment was designed to determine if these seedlings could be reused for indexing.

Most of the indexing of the trees in cherry scion orchards had been done in the greenhouse. With an increase expected in amount of indexing required it is likely that more indexing will have to be done in the field. Thus an experiment was designed to test a new method for field indexing.

Reaction of Shirofugen index plants to buds from trees which previously had given necrotic ring spot negative reactions on P. tomentosa

The trees in the Inter-State Nurseries' cherry scion orchard at Hamburg, Iowa which have been indexed several times with P. tomentosa, were again indexed on this host during the winter of 1957. All trees giving positive reactions were removed from the orchard in the spring. During the summer of 1958, 250 trees in this orchard were indexed on Shirofugen trees located at Hamburg, Iowa. The indexing method used was that described by Milbrath and Zeller (1945) which involved placing buds of suspects on current seasons growth using the T-bud technique. Presence of virus in the bud was indicated by excessive gumming and necrosis of tissues in vicinity of the bud.

Six of 250 trees gave positive reactions for necrotic ring spot virus on Shirofugen. Three of these trees were replacements which had not been previously indexed. Two other positives were Napoleon variety sweet cherries. When these two trees were indexed on P. tomentosa during the winter of 1958 both were found to be necrotic ring spot positive, though symptoms were delayed, not being apparent for nearly six weeks. Such late expression of symptoms may account for their not having been detected in previous indexing with P. tomentosa. Buds from the other positive plant

were inserted in six P. tomentosa plants, with only one of the six showing typical necrotic ring spot symptoms.

These results indicate that P. tomentosa was as effective in detecting necrotic ring spot virus in trees of this orchard as Shirofugen for only one of the trees which gave a positive reaction on Shirofugen failed to be consistently detected on P. tomentosa.

Response of healthy P. tomentosa seedlings which were inoculated with necrotic ring spot virus after defoliation

An experiment was conducted to determine if P. tomentosa seedlings which had been used for indexing and which had given negative necrotic ring spot reactions could be re-used for indexing. In a preliminary experiment seedlings were inoculated at a stage when most of the leaves were mature (which would be the condition of leaves on index plants after use). Of ten plants inoculated, none developed symptoms. Therefore it seemed that if plants were to be reused they probably would have to be defoliated. In early indexing studies Hildebrand (1942) had found that if a rapidly growing peach tree is inoculated with an infected bud, and the stem cut off one node above the diseased bud from 0 to 7 days afterwards, new growth was stimulated on which symptoms developed.

In this experiment new growth formation was stimulated by removing all branches and leaves and cutting back the

stem a few inches (Figures 5 and 6). Four or five days after this treatment each of 18 sources of necrotic ring spot was inoculated into ten defoliated seedlings. This experiment was concurrent with the experiment reported in Table 2. In that experiment plants at bud break were inoculated with the same 18 sources of necrotic ring spot virus. This enabled a comparison to be made between the results from the usual method of indexing and the results from the method under examination. Twenty check plants which were not inoculated were included, ten defoliated and ten nondefoliated.

Symptom observations were made for six weeks after inoculation. A record was kept of the day on which symptoms were first observed in each plant. Symptoms were classified into three groups which were numerically rated: 1 - mottle only; 2 - mottle and necrotic spots; and 3 - mottle, necrotic spots and other necrosis including die-back of terminals. Measurements were made of the width of the three largest leaves on each plant.

These observations are reported in Table 8. All inoculated plants, whether defoliated or not, expressed typical necrotic ring spot symptoms. There was little if any difference in time of appearance of symptoms between defoliated and nondefoliated trees. The defoliated trees had smaller leaves than the nondefoliated. Leaves from uninoculated trees were larger than leaves from inoculated trees.

Figure 4. *P. tomentosa* seedlings. Left to right: uninoculated check, uninoculated check, and inoculated with necrotic ring spot virus

Figure 5. *P. tomentosa* seedlings of Figure 4, defoliated. Left to right: uninoculated check, inoculated after defoliation, and inoculated prior to defoliation

Figure 6. *P. tomentosa* seedlings of Figure 5, four weeks after defoliation. Left to right: uninoculated check, inoculated after defoliation, and inoculated prior to defoliation

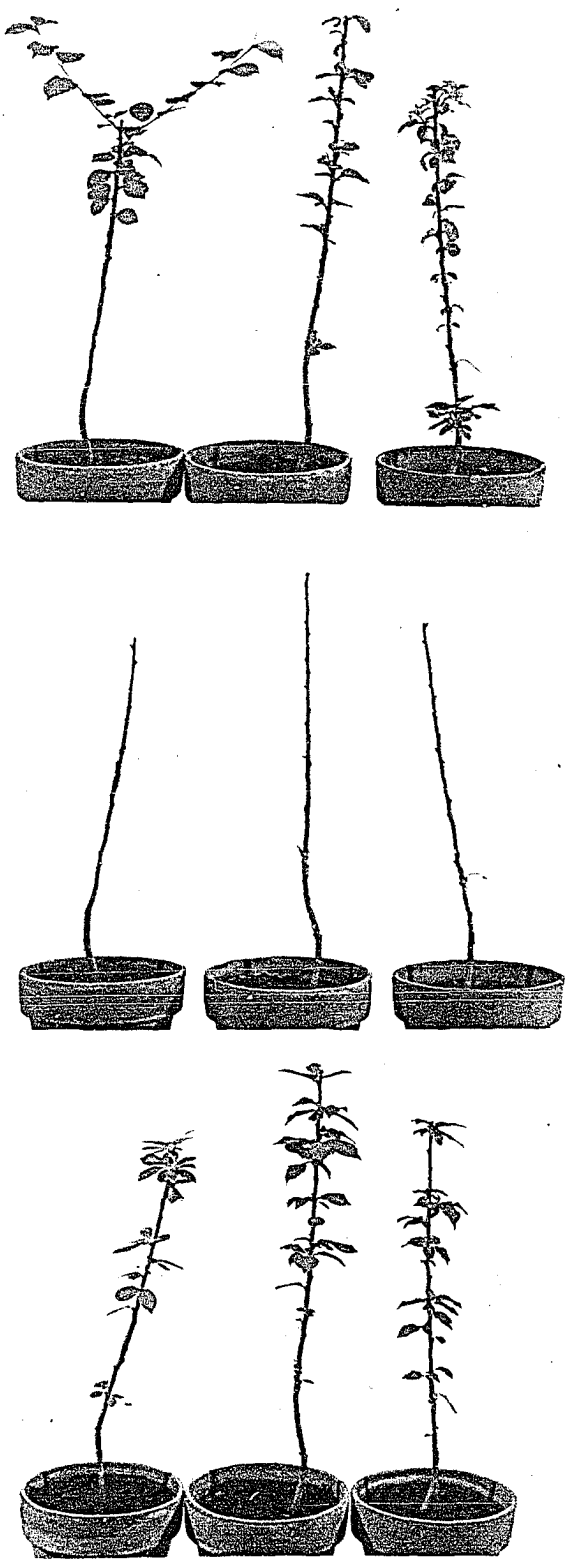


Table 8. Response of P. tomentosa seedlings inoculated with necrotic ring spot virus at bud break and after defoliation. Greenhouse, 1957.

Source of inoculum	No. of inoculated plants which expressed symptoms		Time (days) from inoculation to first appearance of symptoms		Width of leaves (average of largest 3; cm.)		Symptom index[a]	
B-1-12	10		10.2[b]		5.0[b]		2.0[b]	
B-1-12(d)[c]		10		12.6		4.9		1.6
G-2-1	10		12.1		5.4		1.4	
G-2-1(d)		10		14.3		5.1		1.8
G-5-1	10		10.8		5.5		1.7	
G-5-1(d)		10		16.0		5.0		1.5
M-3-17	10		9.9		6.1		1.7	
M-3-17(d)		10		13.1		4.8		1.9
M-6-19	10		9.2		5.2		2.1	
M-6-19(d)		10		12.2		4.8		1.7
M-6-30	10		8.6		6.1		1.9	
M-6-30(d)		10		12.7		4.9		1.9
S-1649	10		13.2		4.2		1.6	
S-1649(d)		10		11.7		4.7		2.1

[a]Symptom classification: mild - 1, moderate - 2, severe - 3.

[b]Each datum is the average of data from 10 plants.

[c](d) = defoliated plants.

Table 8 (continued)

Source of inoculum	No. of inoculated plants which expressed symptoms		Time (days) from inoculation to first appearance of symptoms		Width of leaves (average of largest 3; cm.)		Symptom index[a]	
S-1563	10		9.5		4.9		1.9	
S-1563(d)		10		11.1		4.9		2.1
B-3-23	10		11.1		5.5		1.3	
B-3-23(d)		10		14.2		5.5		1.7
M-7-74	10		18.0		6.1		1.0	
M-7-74(d)		10		17.1		5.9		1.2
B-3-22	10		8.9		5.5		2.1	
B-3-22(d)		10		10.0		3.9		2.1
G-2-7	10		11.1		5.4		1.6	
G-2-7(d)		10		14.4		4.8		1.6
G-20-5	10		14.5		5.3		1.2	
G-20-5(d)		10		13.8		4.2		1.6
S-5105	10		13.5		5.5		1.8	
S-5105(d)		10		12.3		4.2		2.1
HSB-15-7	10		15.1		4.8		1.2	
HSB-15-7(d)		10		14.8		4.8		1.8
B-1-35	10		10.4		5.8		1.2	
B-1-35(d)		10		11.3		4.9		1.8
M-5-74	10		10.2		6.0		1.5	
M-5-74(d)		10		11.2		4.3		1.2

Table 8 (continued)

Source of inoculum	No. of inoculated plants which expressed symptoms		Time (days) from inoculation to first appearance of symptoms		Width of leaves (average of largest 3; cm.)		Symptom index[a]	
Fruit-morency	10		13.6		5.4		1.5	
Fruit-morency(d)		10		15.2		4.6		1.4
None	-		-		8.4		-	
None(d)		-		-		6.3		-
Total			209.9	238.0	106.1	92.5	28.7	31.1
Average			11.6	13.2	5.58	4.86	1.59	1.72

Frequently the defoliated trees had leaves with necrotic tissue which could not be determined as due to virus infection. This made the reading of symptoms more arduous than with non-defoliated trees. However the fact that all defoliated trees inoculated expressed symptoms indicates that P. tomentosa seedlings could be re-used for indexing, especially for routine indexing.

Field response of P. tomentosa seedlings which were inoculated and placed in cold storage prior to placement in field

Greenhouse space is often limited during the period favorable for winter indexing with P. tomentosa. Thus there is a need for indexing methods which would not involve greenhouse use. P. tomentosa has been used in field indexing, buds from suspects being placed on growing plants which were then cut back. Observation for symptoms then being made on the new growth.

A method of field indexing was studied which involved budding of seedlings prior to their placement in the field. Thirty dormant seedlings were removed from cold storage. Twenty of these were inoculated with sources of necrotic ring spot virus, ten with Fruitmorency buds and ten with buds of source B-3-23. Ten plants were kept as checks, five which were not budded, and five which were budded with virus-free budwood. After being budded, these plants were returned

to cold storage; then in late spring after danger of frost had passed, they were lined out in a field plot. Symptoms were observed on June 26, about two weeks after plants had been set in the field. All of the inoculated plants gave necrotic ring spot positive reactions and all of the checks negative reactions.

DISCUSSION

The data show that necrotic ring spot virus is latent in Prunus tomentosa. This virus is also latent in P. cerasus variety Montmorency and Moore and Keitt (1949) made use of this fact in developing their "indirect" method of indexing. Hobart and Buchholtz (1955) in an investigation to determine whether or not the indirect method of indexing could be used to determine amount of necrotic ring spot virus contamination in lots of P. mahaleb seedlings found that this virus is not latent in P. mahaleb. If latent necrotic ring spot virus is present in P. tomentosa seedlings used for indexing, the indexing results might be ambiguous since an inserted bud containing necrotic ring spot virus might not cause symptoms to be induced because of cross protection by the virus in the plant, and since symptoms could develop in a seedling even though an inserted bud was virus free inasmuch as the latency might be incomplete. Fridlund (1954a) found that P. tomentosa seedlings which had been inoculated with some necrotic ring spot sources and subsequently defoliated and reinoculated seldom expressed acute symptoms. Of 13 inoculation-reinoculation combinations only one resulted in plants expressing acute symptoms and these symptoms were not typical. In the observations recorded herein the sources which contained virus recurrent in sour cherry also contained

virus recurrent in P. tomentosa with the exception of source M-6-19. However symptoms were not as severe as the shock symptoms immediately after inoculation. The fact that latency of necrotic ring spot virus may be either complete or partial in P. tomentosa indicates that an accurate determination of virus present in P. tomentosa seedlings used for indexing could not be obtained by using the indirect method to index samples from lots of seedlings. Assurance that seedlings used for indexing are free of necrotic ring spot virus is best obtained by indexing the trees from which seed is harvested to produce seedlings.

Mechanical transmission of necrotic ring spot virus has been accomplished experimentally from Prunus species to herbaceous plants and under special conditions (seedlings with less than eight leaves) from Prunus to Prunus (Fulton, 1957). The contact experiments reported here were designed to examine the possibility of transmission of necrotic ring spot virus from Prunus to Prunus by mechanical means. The results which show that contact periods of less than three days were not sufficient to induce symptom formation in inoculated plants, are not suggestive of mechanical transmission. Gilmer (1954) in a report without data stated that necrotic ring spot infected buds removed 96 hours after inoculation resulted in transmission, but prior removal did not result in transmission. Kunkel (1938) in a contact

experiment with peaches found that the virus of peach mosaic is transmitted in from two to three days, and that the viruses of little peach, peach yellows, and peach rosette were transmitted in from 8 to 14 days. Mechanical transmission of these viruses has not been reported.

Although transmission was occasionally obtained in experiments involving superficial contact of stems and roots, these experiments likewise are not suggestive of mechanical transmission.

Transmission of virus by crude sap inoculations was of especial interest in that in one test all of the 20 plants inoculated expressed symptoms. If one could be assured that it was the sap which was infective and not the leaf tissue in the sap, this could be considered mechanical transmission. However, since the sap was not free of leaf tissues this one instance of transmission cannot be assuredly considered as mechanical. Other experiments indicated that virus in leaf tissue (strips) placed under bark flaps was transmitted to P. tomentosa seedlings.

The indexing with Shirofugen in addition to indicating the effectiveness of prior indexing with P. tomentosa, showed that Shirofugen may be of value for indexing for necrotic ring spot under Iowa conditions.

SUMMARY

Data are presented which show that necrotic ring spot virus is latent in Prunus tomentosa, that buds and bark containing necrotic ring spot virus must be left in contact with healthy cherry trees for three days or longer if symptoms are to develop, and that transmission of necrotic ring spot virus from diseased to healthy plants can occur when roots are grafted, but is not likely to occur when roots or stems are in superficial contact. Other data show that infected leaf tissue placed under bark flaps can infect P. tomentosa seedlings and that necrotic ring spot virus in crude sap placed under bark flaps can be infective to P. tomentosa seedlings. Still other data indicate the effectiveness of previous indexing with P. tomentosa, the feasibility of re-using P. tomentosa plants previously used for indexing, and the effectiveness of a new method of field indexing.

That necrotic ring spot virus can exist in P. tomentosa seedlings in a latent condition was demonstrated by inoculating ten plants with each of 18 sources of necrotic ring spot virus and one year later reinoculating six of the ten, two with the same source, two with a similar source, and two with a dissimilar source. Symptom observations showed that many plants did not express symptoms the second time and that on those which did, symptoms were mild. Necrotic symptoms were rare during the second season, though they were common

after first inoculation of virus-free plants. Similar results were obtained with seedlings which were infected with the 18 sources, defoliated, and reinoculated with source M-6-30 (recurrent) or Fruitmorency.

Studies were made of the length of time inoculum containing necrotic ring spot virus must be left in contact with P. tomentosa seedlings and Gilbert Montmorency trees if necrotic ring spot symptoms are to develop. (1) P. tomentosa seedlings were inoculated when dormant, at bud break, and when buds were open, with dormant buds, open buds, and dormant bark of infected Fruitmorency trees. Inoculum was left in contact with the trees for 1, 2, 4, 8, and 42 days in one experiment and 2, 3, 4, 6, and 8 days in another. Symptoms did not develop under any of the conditions if inoculum was removed within three days. Transmission was seemingly most rapid when trees with buds open were inoculated with dormant buds. (2) P. tomentosa trees at bud break were inoculated with P. tomentosa buds infected with necrotic ring spot virus. Inoculum was removed after 1, 2, 3, 4, 6, 8, and 42 days. No symptoms developed on plants on which inoculum was left for four days or less. (3) Gilbert Montmorency trees at bud break were inoculated with Fruitmorency buds. Inoculum was removed after 1, 2, 4, 8, and 42 days. Transmission occurred in only one case with contact periods of four days or less and in nine cases when contact period was eight days.

Transmission of necrotic ring spot virus from infected to healthy P. tomentosa seedlings was investigated by placing uninjured roots, injured roots, and grafted roots in contact for six weeks. Of thirty pairs of plants, ten in each category, transmission was obtained in only two cases (root grafts).

The possibility of necrotic ring spot virus being transmitted from diseased to healthy plants when stems are in superficial contact for six weeks was examined. Pairs of P. tomentosa seedlings (20) with injured stems and pairs (20) with uninjured stems were held together with budding rubbers. One member of each pair was inoculated at the time of contact. Transmission was obtained in only one case (injured stem). In another experiment stems of seedlings previously infected and showing symptoms were placed in contact with stems of healthy seedlings. All stems were injured prior to contact. Ten pairs were left in contact for seven days, ten for ten days, and ten for 42 days. Transmission was obtained in only one case (ten-day contact period).

Transmission of necrotic ring spot virus to P. tomentosa seedlings by placing infected leaf tissue under bark flaps was obtained in three of 50 cases.

Necrotic ring spot virus in crude sap from young Fruitmorency leaves was transmitted to P. tomentosa seedlings. Sterile cotton soaked in sap was placed under bark flaps which were wrapped with budding rubbers. Transmission was

obtained in all of 20 trials. Similar attempts using leaves of Gilbert Montmorency trees to prepare crude sap and sap free of leaf tissue were unsuccessful.

To determine effectiveness of previous indexing, a cherry scion orchard which had been indexed repeatedly on P. tomentosa seedlings was re-indexed on Shirofugen trees. Only one of 250 trees was shown to contain necrotic ring spot virus which could not be consistently detected by use of P. tomentosa.

P. tomentosa seedlings previously used for indexing and showing negative reactions were tested for possible reuse in indexing. The seedlings were cut back and completely defoliated, then inoculated with each of 18 sources of necrotic ring spot virus. All inoculated seedlings expressed typical necrotic ring spot symptoms. No difference was apparent in the time of appearance of symptoms between plants inoculated after defoliation and plants inoculated at bud break.

A new method of field indexing was tested for possible effectiveness. P. tomentosa seedlings were removed from cold storage during the winter and inoculated with sources of necrotic ring spot virus, ten with Fruitmorency and ten with B-3-23. Ten checks were not inoculated. These plants were returned to cold storage and in the spring set in the field. All inoculated plants expressed typical symptoms and none of the checks were infected.

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